Spatiotemporal mechanisms of root branching

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Abstract

The fundamental tasks of the root system are, besides anchoring, mediating interactions between plant and soil and providing the plant with water and nutrients. The architecture of the root system is controlled by endogenous mechanisms that constantly integrate environmental signals, such as availability of nutrients and water. Extremely important for efficient soil exploitation and survival under less favorable conditions is the developmental flexibility of the root system that is largely determined by its postembryonic branching capacity. Modulation of initiation and outgrowth of lateral roots provides roots with an exceptional plasticity, allows optimal adjustment to underground heterogeneity, and enables effective soil exploitation and use of resources. Here we discuss recent advances in understanding the molecular mechanisms that shape the plant root system and integrate external cues to adapt to the changing environment.

Molecular mechanisms determining rhizotaxis

The earliest events that determine the position of the future branching site occur in the basal meristem (Glossary) of the primary root. Here, oscillatory gene expression patterns along with a locally increased response to the plant hormone auxin prime protoxylem cells(Glossary), thereby defining the pre-branching site [1, 2]. Although the molecular mechanisms underlying this complex oscillatory pattern are not fully understood, recent studies provide important insights into pathways that contribute to this oscillation-based root branching (Figure 1A).

A screen for chemical compounds affecting root branching patterns provided unexpected hints on the role of the root cap in rhizotaxis (Glossary). A non-auxin-like compound, designated naxillin, was identified due to its dramatic impact on root branching. A prerequisite of naxillin function is the INDOLE-3-BUTYRIC ACID-RESPONSE3 (IBR3) driven conversion of the indole-3-butyric acid (IBA) to a bioactive auxin, indole-3-acetic acid (IAA) in the root cap [3, 4] that has been considered to play an important role as auxin supplier necessary to pre-set the regular branching pattern [5]. Furthermore, the root cap is not only a source of auxin, but also it functions as an active component of the oscillatory mechanism. Programmed cell death (PCD) of the lateral root (LR) cap cells that occurs when entering the elongation zone displays robust oscillations. Consequently, auxin pulses are released to surrounding root tissues and define the LR spacing along the main root. It is also tempting to speculate that turnover of the root cap around the very tip of the growing root may perceive and transmit environmental signals to coordinate the primary root growth with root branching to optimize the uptake of water and nutrients from the soil [5]. Disruption of carotenoid biosynthesis has been found to cause a significant reduction in the pre-branching site numbers (Glossary), implying that this pathway

plays a role in rhizotaxis. Several carotenoid biosynthesis genes have been detected in the proximal, but excluded from the priming (oscillatory) zone of roots (Glossary), suggesting that a non-cell-autonomous mechanism might be involved in the carotenoid-mediated control of the LR pre-patterning [6]. Another recently uncovered pathway with a significant impact on the root branching pattern involves regulation of symplastic connections among cells and tissues. Prior to and during the early phases of LR initiation (LRI) (Glossary), all cells are symplastically connected to the pericycle (Glossary). Interference with symplastic connectivity dramatically affects the LR branching pattern and leads to multiple initiation sites in close proximity [7]. However, how a reduction in symplastic connectivity acts on early LR events and which are the mechanisms underlying the LR spacing control need to be resolved.

Unlike the above discussed endogenously driven clock mechanisms, hydro-patterning integrates water availability as an external signal to shape the root system. Roots of several plant species distinguish between liquid and air environments and adapt early LRI toward a damp side. High water availability upregulates the *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1* (*TAA1*) and *PIN-FORMED 3* (*PIN3*) auxin efflux carriers, resulting in local LR formation-triggering induction of auxin biosynthesis and transport [8]. Similarly to hydro-patterning, primary root bending caused by deflections from obstacles or gravitropic bending is an environment-driven mechanism that regulates rhizotaxis. Local accumulation of auxin at the convex side of curves promotes LRI, thus directly affecting the root branching pattern [9–11]. Interestingly, interference with the activity of members of the PLETHORA transcription factor family in *Arabidopsis thaliana* leads to defects in both rhizo- and phyllotaxis. Thus, mechanisms controlling shoot and root lateral organ positioning might be shared [12].

The acropetal architecture of the root system in *Arabidopsis* results from initiation events limited to a restricted zone behind the root tip, also designated a developmental window (Glossary) [13, 14]. To stabilize and maintain the typical root branching pattern, LRIs outside of the developmental window are suppressed [15]. Interference with the hormonal activity of cytokinin increases the initiation of ectopic primordia near exiting LRs, suggesting that a cytokinin-mediated pathway might act as a positional cue to stabilize the LR spacing pattern and to constrain ectopic formation of lateral branches [16–18]. Besides cytokinin, *ARABIDOPSIS CRINKLY4* (*ACR4*), identified in a cluster of genes induced early upon LRI, appears to be an important rhizotaxis regulator [19]. *ACR4* encodes a plasma membrane-localized receptor-like kinase and its loss-of-function leads to the formation of ectopic and/or fused primordia. Cytokinin does not counteract LR spacing defects in *acr4* mutants, implying that cytokinin and *ACR4* act independently to restrict ectopic root branching [20].

Although various mechanisms have been proposed for the establishment and maintenance of the root branching pattern, they are not mutually exclusive, but their inputs might be constantly integrated to shape root branching patterns in response to environmental stimuli [21].

Role of the pericycle–endodermis interaction in launching the LR developmental program

Pathways that predetermine the root branching pattern generate signals that are directed toward xylem pole pericyle cells (Glossary), trigger their specification to founder cells (FCs) (Glossary) and thereby initiate LRs developmental program (Figure 1B). Accumulation of auxin and activation of the transcriptional regulator *GATA23* are the earliest hallmarks of FC establishment after priming [22, 23]. However, the following steps are scarcely understood. How is the local

auxin maximum formed in the few distinct pericycle cells and which mechanisms underlie the transition from FC to formative divisions to initiate the LR developmental program? Auxin reflux mediated by PIN3 reinforces the auxin flow from the endodermal to the pericycle cells, enabling them to reach the auxin threshold required for FC specification [24], hinting at a specific function of the endodermis during early LRI phases. Indeed, detailed monitoring of the endodermal cells adjacent to FCs revealed an intriguing regulatory role for this tissue. During early LRI, the endodermal cells next to FCs lose volume, change shape, and release their tight junction-like diffusion barrier to allow FC to expand and undergo formative divisions giving rise to LR primordia (LRP) [25]. A local inhibition of auxin responses in the endodermis restricted strongly the primordia initiation, indicating that this endodermal feedback is auxin dependent [25–27]. Later, details were provided on the endodermis-pericycle interaction that governs early LRI [28]. Single-cell ablation experiments revealed that endodermis elimination can substitute for the auxin-dependent activation of the meristematic activity in the pericycle. However, auxin was indispensable to determine the proper orientation of the cell division plane during the subsequent divisions and, thus, to launch the LR developmental program. Based on these findings, a revised model for the early LRI has been proposed that recognizes spatiotemporally distinct dual auxin functions. In the endodermis, auxin is required to release constraints arising from cell-to-cell interactions that compromise the meristematic activity of the pericycle, whereas, in the pericycle, auxin defines the cell division plane for initiation of the LR developmental program [28].

The two spatiotemporally distinct functions of auxin that trigger and delineate the LR organogenesis program raise a number of intriguing questions, such as what is the molecular basis of the auxin-driven morphogenetic modulations of endodermis that permits adjacent

pericycle cells to re-enter the cell cycle and which molecular pathway downstream of auxin defines the formative divisions of FCs. Recent reports addressing some of these questions provide the first clues on the possible molecular players and underlying mechanisms. For example, the increase in cell volume of pericycle cells re-entering the cell cycle after ablation of adjacent endodermal cells suggests that the endodermis might restrain the pericycle cell cycle progression mechanically. The auxin-controlled expression of several cell wall-remodeling genes, specifically in endodermal cells above initiated primordia [14] and the severely compromised LRI by the endodermis-specific auxin signaling suppression [25, 26] support a role for auxin in releasing mechanical constraints exhibited by the endodermis. Noteworthy, endodermal cells are impregnated with hydrophobic substances (Casparian strips) that restrict the apoplastic water flow toward the inside tissues [29]. Hence, auxin might also regulate pathways that impact on water transport and turgor-driven mechanisms, as corroborated by the hydro-patterning model [8]. An interactor of the LR-promoting ARABIDILLO proteins produced specifically in the endodermal cells overlying early LRP is an emerging candidate for the auxin-induced feedback loop that is stimulated in a few endodermal cells to control the LRI [30].

In contrast to the endodermis, in dividing pericycle cells, auxin seems to be primarily required to define formative anticlinal cell divisions that delineate the LR organogenesis. Among the cellular components decisive for cell division plane orientation, the microtubule cytoskeleton is known to have a dominant function. Analysis of a mutant lacking the microtubule-severing factor KATANIN 1 (KTN1) indicates that a dynamic microtubule cytoskeleton might be an important component of the auxin-driven framework that specifies formative division plane orientations in pericycle cells [28]. How these spatially and temporary distinct auxin-dependent

activities are integrated into known Transport Inhibitor Response (TIR)-Auxin Response Factor (ARF)-Auxin/IAA signaling modules that control LR organogenesis needs to be resolved [31].

Patterning, organogenesis, and emergence of LRs

After LRI, organogenesis proceeds via coordinated cell division and differentiation patterns. Several studies have focused on the identification of the main rules governing LR morphogenesis in *Arabidopsis* [32–34]. Detailed real-time imaging analyses revealed that LR organogenesis follows few basic patterning characteristics such as (*i*) LRs arise from a variable number of FCs, of which some take a dominant role; (*ii*) the first asymmetric division of FCs is tightly regulated; (*iii*) later divisions in the primordia do not follow a strict order, and (*iv*) interaction with the overlying tissue is instrumental for the primordia morphogenesis [33, 34].

LR morphogenesis is tightly linked with a graded auxin distribution [35]. Defects in auxin transport mediated through auxin influx and efflux carriers of the AUX/LIKE-AUXIN (LAX), PIN, and ATP-binding cassette subfamily B/multi-drug resistance/P-glycoprotein (ABCB/MDR/PGP) families result in abnormal LR patterning [36]. Recently, several transcriptional regulators have been identified that control and fine-tune the expression of auxin transporters during LR organogenesis (LRO). For example, *PIN3* expression during early LR formation is specified by an ARF7-FOUR LIPS (FLP) feed-forward circuit [37]. Also, FLP and its homolog MYB88 determine spatiotemporal patterns of the *PIN3* and *PIN7* transcription to coordinate the LR responses to gravity [38]. XAL2/AGL14, an auxin-regulated MADS box transcription factor might, through direct control of the *PIN1* and *PIN4* transcription, contribute to LR patterning [39]. Furthermore, the CYTOKININ RESPONSE FACTOR 2 (CRF2), CRF3,

and CRF6 that act as direct transcriptional regulators of *PIN1* and *PIN7* emerge as potential integrators of various signals, including other hormones and environmental inputs to fine-tune LRO [40, 41] (Figure 1B).

Precise coordination of the auxin flow during development of LRP depends largely on the polar membrane localization of the auxin transporters. Factors and mechanisms that directly impact on primordia morphogenesis via control of subcellular trafficking and targeting of auxin transporters have been reported. A *trans*-Golgi network-localized factor 1 (TNO1) that controls the vacuolar protein transport, has been found to regulate the cellular auxin transport during LR emergence through modulation of trafficking of auxin transporters [42]. DIAGEOTROPICA, a cyclophilin A ortholog, previously identified for its role in the coordination of proper primordia division pattern, has been implicated in the control of the PIN1 plasma membrane localization [43]. By means of a quantitative phospho-proteomics approach to reveal early signaling events in auxin-triggered LR organogenesis, PIN2 and ABCB/MDR/PGP as well as SORTING NEXIN 1 (SNX1), a regulatory component of the vacuolar trafficking machinery, were identified. Thus, the auxin-driven LRO involves rapid signaling, such as phosphorylation that might also target protein subcellular trafficking in controlling LR development [44]. Furthermore, cytokinin-driven modulation of endocytic trafficking of PIN proteins arises as a novel mechanism of rapid regulation of auxin flow during LR organogenesis. Cytokinin enhances PIN1 depletion at specific polar domains and, thereby, directs the auxin flux toward the tip of primordia, promoting their development [45] (Figure 1C).

The auxin gradient is interpreted through downstream genes and regulatory networks that control LR organogenesis. Although several genes involved in this process have been identified in *Arabidopsis*, how these components form a functional regulatory network is largely unknown.

To decipher the gene regulatory network that control LRO a time-delay correlation algorithm (TDCor) has been developed from a time series of transcriptomic data. The predicted network revealed that the interaction of two mutually inhibitory ARF7 and ARF5 modules, previously identified to control LR organogenesis [31], generates the bifurcation between two cell fates, leading to the specification of the flanking and central zones of developing LRP [33].

An important auxin-controlled mechanism of primordia patterning is mediated via aquaporins, which are membrane channels that facilitate water movement across cell membranes. Both aquaporin isoforms - plasma and tonoplast membrane intrinsic proteins (PIPs and TIPs), were found to regulate the root tissue water transport that is required during LRP morphogenesis and emergence [46, 47]. Transcriptomic analysis of LR development revealed that auxin-mediated LR emergence is under the control of the circadian clock and that disrupting the clock function impairs this developmental process. Interestingly, a local re-phasing of the circadian clock observed in the vicinity of initiated primordia has been proposed as a mechanism that specifies hydraulic conditions distinct to other root tissues to facilitate LRP emergence [48].

As discussed above, interaction with overlaying tissues rather than the precise patterning of divisions within primordia, is most critical for LRP morphogenesis and the emergence process [26, 34]. Work focused on communication between primordia and surrounding tissues demonstrated that auxin produced in the primordium is transported toward the adjacent tissues where it triggers cell separation by inducing both the auxin influx carrier *LAX3* and cell wall-remodeling enzymes [14]. Interestingly, *LAX3* expression is restricted to a few cells in the primordia-surrounding cortex. Mathematical modelling and experimental observations demonstrated that the *LAX3* expression pattern is dependent on the PIN3 mediated auxin efflux, which is required to prevent the transient expression of *LAX3* in multiple cell files [49].

Recently, core components of this auxin-driven molecular path have been uncovered and the sequentially acting ARF7, LATERAL ORGAN BOUNDARIES DOMAIN 29 (LBD29), and LAX3 have been demonstrated to coordinate cell separation and LR emergence [50].

Although priming, initiation, and formation of LRs are considered to be the major determinants of the root system architecture, the importance of mechanisms that determine vectorial growth of emerging LRs and define gravitropic setpoint angle (GSA) of lateral organs cannot be neglected. GSA has a direct impact on the radial expansion of the root system and, thus, represents an essential strategy for soil exploitation. Recently, the molecular principles of GSA have been assessed, demonstrating that auxin, through TIR/AUXIN SIGNALING F-Box (AFB)-Aux/IAA-ARF-dependent auxin signaling and tight regulation of PIN-mediated auxin transport, steers GSA formation and, thus, limits the (positive) orthogravitropism in LRs [51, 52].

Conclusions and perspectives

Plants as sessile organisms are permanently attached to their germination site. Thus, the important part of their survival strategy is their ability to sense and respond to various environmental stimuli. The interaction with soil is governed by the root organs that constantly detect fluctuations in physical and chemical properties of the surrounding environment. How are these various external signals perceived and integrated into endogenous networks and circuits that control the root system establishment is one of the great challenges of future research. First molecular insights into mechanisms that underlie the root interaction with environment have been reported. For example CRF2 and CRF3 have been demonstrated to function in the LR adaptation to cold stress [41]. Oxidative-stress induced reactive oxygen species (ROS) facilitate

LR outgrowth by promoting cell wall remodeling in adjacent tissues [53], but they also seem to impact on the redistribution of the plasma membrane localized aquaporins [54]. The stress hormone abscisic acid (ABA) that induced LR growth quiescence, is controlled via the PYL8 and PYL9 receptors [55] and inhibition of ABA signaling and auxin homeostasis by WRKY46 might modulate osmotic stress-dependent LR hampering [56]. Several studies assessed molecular mechanisms underlying root system response to fluctuations in nutrient availability. The nitrate transporter NRT1.1 exhibits capacity to transport auxin and prevents LR emergence under low nitrate [57]. In nitrate-rich medium PIN mediated auxin efflux and subsequent cell cycle activation were found to enhance LRI [57, 58]. How far these mechanisms are conserved across various plant species is another important question to be addressed.

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●● of outstanding interest

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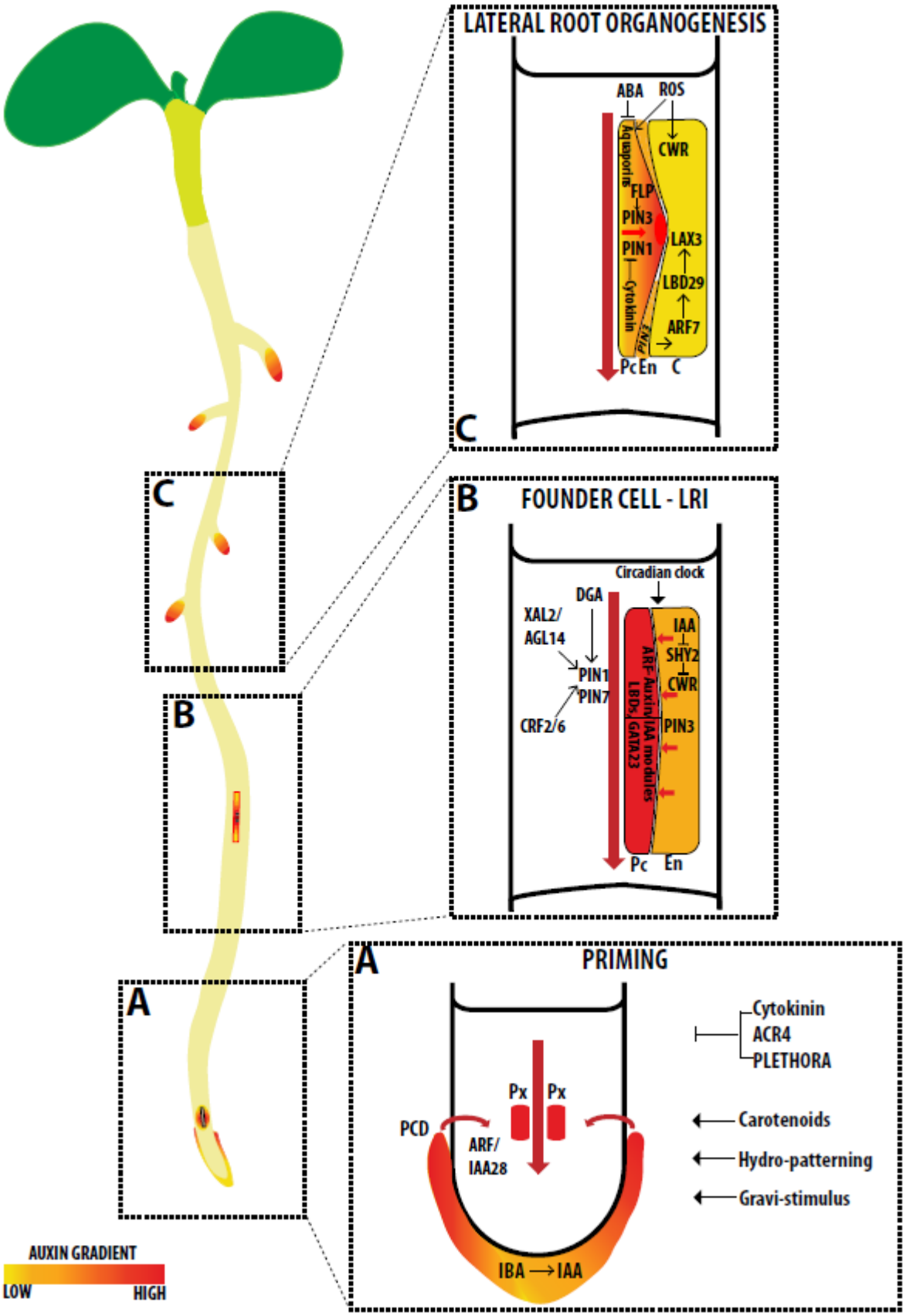
LATERAL ROOT ORGANOGENESIS
ABA
ROS
Aquaporins
CWR
FLP
PIN3
PIN1
Cytokinin
LAX3
LBD29
ARF7
ENP
Pc En C
C
C
B
FOUNDER CELL - LRI
Circadian clock
DGA
XAL2/
AGL14
PIN1
PIN7
CRF2/6
IAA
SHY2
CWR
PIN3
ARF-Auxin/IAA modules
LBDs, GATA23
Pc
En
B
A
PRIMING
Px
Px
PCD
ARF/
IAA28
IBA → IAA
Cytokinin
ACR4
PLETHORA
Carotenoids
Hydro-patterning
Gravi-stimulus
A
AUXIN GRADIENT
LOW
HIGH

Figure 1. Spatiotemporal control of the root branching pattern and lateral root organogenesis. Priming of protoxylem cells (Px) in the root basal meristem (A) defines root branching pattern and pre-determine positioning of founder cells (FCs) in the pericycle (Pe) (B). After specification FCs undergo formative divisions and initiate lateral root organogenesis program (C).

(A) Overview of pathways contributing to establishment of the root branching pattern. In the root cap IBA is converted to auxin and transported towards basal meristem where it is required for priming. Programmed cell death (PCD) of lateral root cap cells generates pulses of auxin that is transduced via IAA28 auxin signaling module to promote priming. Carotenoid biosynthesis, root bending in response to gravi-stimulus, as well as water availability (hydro-patterning) farther fine tune root branching pattern. Cytokinin signaling, ACR4 and PLETHORA mediated signaling limits initiation of lateral roots in ectopic positions and stabilize root branching pattern. (B) PIN3 mediated auxin reflux enhances auxin accumulation in FCs and promotes their transition to lateral root initiation (LRI) phase via activation of specific IAA14-ARF7, ARF19, and BDL/IAA12-MP/ARF5 modules and downstream acting components. SHY2/IAA3 mediated signaling coordinate morphogenetic modulation of endodermis to permit LRI. (C) Cytokinin enhances PIN1 depletion at specific polar domains and promotes formation of auxin maxima to control LR organogenesis. Auxin-dependent primordia patterning rely on the regulation of hydraulic properties of cells through modulation of aquaporins expression. Furthermore, auxin via PIN3 - LAX3 mediated transport is released to adjacent endodermis (En) and cortex (C) cells where it coordinates expression of cell wall remodeling (CWR) genes thereby facilitating LR emergence. Reactive oxygen species (ROS) targets CWR and aquaporins to promote primordia emergence. Contrarily abscisic acid (ABA) inhibits the process. Red arrow indicates direction of auxin flow in the primary root and LRP.